REVIEW ON EFFECT OF PRETREATMENT ON
DIGESTIBILITY OF CELLULOSIC MATERIALS

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INTRODUCTION

Cellulosic residues are a renewable resource of which over a billion tons are generated annually in this country (1, 2). These residues encompass materials such as agricultural residues, residues from wood harvesting and processing, and cellulosic materials found in municipal wastes. While the utilization of these materials as potential sources of liquid fuels has received much attention, it should not be forgotten that some of these materials are also suitable for protein production.

Forages (grasses) and agricultural residues (such as corn residue, sugarcane bagasse, and straws) have value as animal feed. Cellulosic materials provide bulk as well as fiber (cellulose and hemicellulose). The fiber is partially digestible by microorganisms in ruminant digestive systems and provides some of the animal's energy needs which would otherwise be obtained from grain.

THE STRUCTURE AND COMPONENTS OF CELLULOSIC RESIDUE

Cellulose is a linear polymer of D-anhydroglucopyranose units linked by 1-4-glucosidic bonds, with a degree of polymerization ranging from 15 to 10,000-14,000. Several models for cellulose fine structure have been proposed (3). A folded chain model has been proposed by Chang (4)

According to the Chang model, a cellulose molecule folds back and forth on itself to form a "platellite", the smallest structural unit of cellulose with the approximate dimensions of 1000x39x6A (1000 = Leveling off degree of polymerization). Consequently, several platellites are packed to make up the crystallite of cellulose, the structure of which measures 35 x 30 A in cross section and about 500 to 1000 A along the axis for native cotton cellulose. Corn residue contains about 30% cellulose by Van Soest fiber analysis (5).

Hemicellulose is a short-branched-chain heteropolymer consisting primarily of xylose and small quantities of glucose, galactose, mannose, and arabinose, as well as uronic acids of glucose and galactose. The degree of polymerization is usually less than 200. About 30% hemicellulose is present in corn residue, mainly composed of xylose with some arabinose and glucose and uronic acid as determined by liquid chromatography of sulfuric acid hydrolysates of corn residue (6).

Lignin is a complex three-dimensional polymer arising from an enzyme-initiated dehydration polymerization of three primary precursors: trans-3-Coumaryl alcohol, trans-coniferal alcohol and trans-sinapyl alcohol (7). The schematic formula for spruce lignin has been reported by many researchers (8, 9, 10). The major components are reported to be an arylglycerol-β-aryl

0005-5092/81/5501-0207$02.00
* The American Institute of Chemical Engineers, 1981

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structure, a phenyl coumaran structure, and a biphenyl structure (8). Corn residue is about 10% lignin.

The cellulose, hemicellulose, and lignin in corn residue and other native cellulosic materials exist in a structure which makes the hemicellulose easy to hydrolyze. The cellulose crystalline structure and the lignin which surrounds it forms a "seal" and protects the cellulose from easy hydrolysis.

**CHEMICAL AND PHYSICAL TREATMENT OF CELLULOSIC MATERIAL**

An excellent review of this subject has been published by Millett, Baker, and Satter (11, 13). The basic goal of chemical and physical treatment of cellulosic material is to alter the fine cellulose structure and extensively break up the lignin-carbohydrate complex so that carbohydrate in cellulosic material can be utilized by bacteria in the rumen. Another reason for physical treatment is to increase surface area and decrease crystallinity, and consequently, to increase the effective cellulose concentration. Chemical treatment includes NaOH, NaH, SO₂, NaClO₂, CIO₂, pulping, and more recently, CMCs (caustic metal cellulose swelling). Physical treatments include grinding, irradiation, and steam pretreatments (14, 35).

**Chemical Treatment**

**NaOH Treatment.** NaOH treatment of native cellulosic fiber causes extensive swelling and leads to formation of cellulose II, with a degree of crystallinity of approximately 60% (15). The mechanism of swelling proposed by Chedin and Marsaudon (16) involves an equilibrium reaction between pairs of solvated ions and the cellulose as shown below.

\[
\text{Na}^+\text{OH}^- + n\text{H}_2\text{O} + R_\text{cell}(\text{OH})_3^3 \rightarrow n\text{OH}^- + R_\text{cell}(\text{OH})_3^6 + 3\text{H}_2\text{O}
\]

Chedin and Marsaudon also found that, for the value of n less than 3, which corresponds to a 4% NaOH concentration, the reaction can be written:

\[
R_\text{cell} + \text{NaOH} \rightarrow R_\text{cell}O\text{Na}^+ + \text{H}_2\text{O}
\]

In the dry process of straw treatment, a minimum of 20% NaOH is left on the straw.

**IVMUD (in vitro dry matter digestibility)** increases from 35% to 82% for wheat straw, and from 5% to 50% for poplar wood after treatment. Animal feeding trials have shown that sheep can tolerate treated wheat straw containing 2% residual NaOH when mixed with corn silages or ground alfalfa hay, or when neutralized with acetic acid (17). Higher levels reduced digestibility possibly because of an increased rate of passage, and/or a decreased rate of fiber digestion, which is possibly affected by a chloride anion deficiency (18).

The primary effect of NaOH treatment is postulated to be the saponification of intermolecular ether bonds. This is thought to promote the swelling past the dimensions caused by water and facilitates the penetration of enzymes and microorganisms into the cellulose fine structure (19). Klopotenstein (18) et al. reported that the modes of action of NaOH treatment include (1) solubilizing the hemicellulose, (2) increasing the extent of cellulose and hemicellulose digestion, and (3) increasing the rate of cellulose and hemicellulose digestion.

**NH₃ Treatment.** Liquid NH₃ exerts a strong swelling action on cellulose and leads to a phase change in the crystal structure from cellulose I to cellulose III (19, 20). The optimum conditions for NH₃ treatment of barley straw have been suggested by Møge and Thomsen (2). Maximum in vitro digestibility is obtained with 2.6% NH₃, 62°C and 4 days incubation, or 5.9% NH₃, 30°C and 3-7 days incubation. Treatment of aspen dust with anhydrous liquid or gaseous NH₃ provided a substantial increase in IVMUD from 33% to 47% (22). The digestibility of NH₃-treated aspen is still 6% lower than that of NaOH-treated aspen.

It is believed that ammoolysis of glucuronic acid ester cross linkages makes the carbohydrate more accessible to rumen bacteria. The study of chemical changes in ammoniatiated wood indicate that the major reactions such as ammoolysis of the ester, lactone, and acetal bonds, and depolymerization of hemicellulose and lignin, alter properties of wood (23).

**Delignification** (24, 25, 26, 27, 28, 29). Since it is known that lignin is a major block to the utilization of cellulosic residue, the processes of SO₂, NaClO₂, and pulping seem to be good candidates for disrupting the lignin-carbohydrate complex. Although complete delignification is not required to prepare a
nutritionally acceptable feedstuff, the cost for even partial delignification is still relatively expensive. However, an attractive approach seems to be SO₂ vapor treatment followed by neutralization with ammonia, since an increase in protein equivalence as well as an increase in digestible energy is obtained.

**CMCS Treatment (6, 30, 39).** A ferric sodium tartrate complex in NaOH solution exerts a high dissolving power for cellulose while NaOH alone primarily swells cellulose. The aqueous alkaline complex of iron and sodium tartrate has been used for viscosity measurement in cellulose chemistry for many years (31, 32, 33, 34).

The CMCS complex has a ratio of Fe³⁺: tartrate:NaOH of 1:3:6. The reaction of this 1:3 complex (ferric to tartrate) with cellulose results in a new complex formation through the association of 1:3 complex with the C₂ and C₆ hydroxyl groups in the glucopyranose unit. During the dissolution of cellulose, the 1:3 complex tends to expand the mole ratio to 1:4.5 by abstracting hydroxyl groups of tartrate. This action of expansion leads to disruption of cellulose structure and, consequently, formation of cellulose solution. Such a solvent may have potential for increasing digestibility and is currently under investigation in our laboratory.

**Physical Treatment**

**Steam Treatment (12, 35, 36, 37).** Steam treatment usually exhibits a strong species response for the type of biomass used. The steam process involves the cleavage of acetyl groups to give an acidic medium which then catalyzes partial hydrolysis of the fiber giving digestible sugars. Masonex®, obtained from the hemicellulose portion of wood, is a solution of such sugars which is commercially available as a wood-carbohydrate feed additive. This hemicellulose extract of wood can be used as a nutritional ingredient in feedstuff and also as a binder in making pellet feeds. Recently, tannin-like phenolic compounds have been found in Masonex which are biologically active. The potential value of these components is now being studied (38).

**Grinding.** Grinding results in small particles and consequently high surface areas for the cellulosic material. Vibratory ball milling (40, 41) and differential speed two-roll milling (42) enhance the susceptibility of cellulose to bacterial and enzymatic attack. The response to milling is also quite species selective (43). Ball milling may be impractical in commercial production due to high processing cost. Moreover, the finely ground particles tend to form a slurry which passes through the rumen more quickly and leads to a decrease in in vivo digestibility (44).

Research comparing hammer milling and disk defiberizing on the fermentability of ryegrass straw indicated that disk defiberized straw is significantly more susceptible to in vitro digestibility than hammer-milled straw. Scanning electron microscopy indicated a higher surface area for defiberized straw than for hammer-milled straw (45).

**Irradiation.** Irradiation yields extensive bond cleavage on cellulose at a dosage level of 10⁸ rad, thus improving digestibility of cellulosic material by rumen microorganisms. However, strong species response and the high energy costs hinder practical application.

**DIGESTION IN THE RUMEN**

The utilization of cellulosic residues by the rumen involves a number of different micro-organisms. There are various cellulolytic bacteria (isolated and grouped as described by Hungate (46)). In addition, there are protozoa in the rumen which utilize the bacteria as food (47) and methanogenic bacteria which generate methane from formate, H₂, and CO₂. The organisms found in the rumen fall into four categories: (1) short rods and coccobacillus-like cells, gram (+): (2) long rods, gram (+): (3) gram (-) cells; and (4) gram (+) cells (48), where the predominant types of cells are gram (+). Various bacteria have been reported to be responsible for digesting cellulose in vitro. Bacteroides succinogenes. Ruminococcus has been shown to almost completely utilize hemicellulose in flax and corn hulls as well as xylan. In comparison, Bacteroides succinogenes, appears to be a poor utilizer of Xylans (49).

The products of hemicellulose fermentation by rumen micro-organisms are acetic, propionic and butyric acids with approximately equal proportions of acetic and propionic acid being formed.
Lignin is usually regarded as indigestible in ruminants, yet the disappearance of lignin has been reported in numerous papers [50, 51, 52, 53, 54]. Gaillard and Richards [55] have found that the cell-free rumen liquor of a steer on a diet of spear grass contains macromolecular substances. Fractionation of these substances on Sephadex G-100 showed carbohydrates and lignin-derived compounds to be present. It was suggested that the formation of a soluble carbohydrate-lignin complex by the action of rumen microorganisms on the spear grass may account for the apparent digestion of about half of the total lignin intake.

SUMMARY

The improvement of digestibility of cellulosic materials by rumen microorganisms has the potential of improving the availability of animal protein. This is especially true in third world countries where a significant portion of feed for ruminants is derived from forages (i.e., cellulosic materials). The research in this area indicates that cellulose utilization by ruminants is a complex subject. Hence, research on improving digestion of cellulosic materials requires consideration of the cellulose structure, the physical and chemical changes upon pretreatment of cellulosic materials and the heterogeneous nature of microbial systems which utilize cellulosic materials in ruminants.

LITERATURE CITED


